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Phylogeographic patterns and demographic history of *Pomacea canaliculata* and *Pomacea maculata* from different countries (Ampullariidae, Gastropoda, Mollusca)

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Abstract

Invasive species of *Pomacea* snails are of growing concern when it comes to the conservation of global biodiversity. *Pomacea canaliculata* has been listed among the world's 100 worst invasive species. In this work, phylogeographic patterns and the demographic history of *P. canaliculata* and *P. maculata* from different countries were analyzed using mtDNA cytochrome c oxidase subunit-I (COI) sequences. The results showed that *P. canaliculata* and *P. maculata* had high genetic diversity, significant genetic differentiation, limited gene flow and stable population dynamics among different countries. Genetic diversity of *P. canaliculata* was higher than *P. maculata*. Our study will also provide important information for the effective prevention and control of the spread of *Pomacea* snails.

Keywords

phylogeographic patterns, demographic history, Pomacea canaliculata, Pomacea maculata

Introduction

Biological invasions are considered one of the highest causes of biodiversity loss on a global scale (Vitousek 1997; Ricciardi 2007; Thomaz et al. 2015). They are not only a major threat to the loss of native biodiversity and the structure and function

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of ecosystems, but also seriously affect social and economic development and human health. Freshwater ecosystems are especially vulnerable as a result of widespread human disturbance (Havel et al. 2005; Turak et al. 2016). Invasive bivalves are among the most successful invasive species in freshwater ecosystems (Ricciardi 2007; Oliveira et al. 2011; Paschoal et al. 2015; Ng et al. 2018), which typically comprise r-selected species which adapt to colonize a wide range of aquatic environments and exhibit high fecundity, rapid growth, and broad physiological tolerance to several abiotic factors (Hayes et al. 2015; Nakano et al. 2015). Such characteristics allow them to become dominant species (Karatayev et al. 2007).

Pomacea (Gastropoda: Ampullariidae) is an edible large freshwater snail native to the Amazon River basin of South America (Cowie 2002). It was introduced as the apple snail to many countries of east and southeast Asia (China, Japan, Philippines, Korea, Vietnam, Thailand, Cambodia, Singapore, etc.; Cowie 2002; Joshi and Sebastian 2006), the continental USA (Alabama, Arizona, California, Florida, Georgia, Texas; Rawlings et al. 2007), partial areas of Oceania (Papua New Guinea, New Zealand; Hayes et al. 2008), parts of Europe (Spain; Andree and López 2013), and some Pacific islands, notably the Hawaiian Islands (Tran et al. 2008) due to it having a high nutritional value of rich protein, carotene, vitamins, and minerals. However, *Pomacea* snails' economic potential was over-estimated because consumers disliked the snails' taste and texture, but of the many, mostly small, aquaculture operations that arose, relatively few persisted and the local market failed to sell this snail (Cowie 2002; Hayes et al. 2008; Lv et al. 2013) and led people to give up farming *Pomacea* snails. Subsequently, these snails became pests of wetland rice and other crops, causing massive economic losses due to their wide adaptability to the freshwater habitat combined with a high fecundity (Cowie 2002; Rawlings et al. 2007; Hayes et al. 2008). However, their ecological impacts are more difficult to estimate as they also continue to spread into nonagricultural wetlands of many countries in the current period (Levin 2006; Wood et al. 2006; Rawlings et al. 2007). At the same time, *Pomacea* snails have been associated with the decline of native snails, and the loss of macrophytes and freshwater bryozoans in natural wetlands, resulting in shifts in ecosystem state and function (Carlsson et al. 2004; Carlsson and Lacoursière 2005; Wood et al. 2006). Additionally, *Pomacea* snails can affect human health, having driven the emergence of human eosinophilic meningitis in China: they have been identified as a major intermediate host of *Angiostrongylus cantonensis*, the rat lungworm (Lv et al. 2008, 2009a, b). *Pomacea canaliculata* was therefore listed among the world's 100 worst invasive species (Lowe et al. 2000).

There have been many studies on *Pomacea*, such as distribution (Rawlings et al. 2007), disease (Lv et al. 2009a, b; Lv et al. 2011), phylogenetic (Rawlings et al. 2007; Hayes et al. 2008; Hayes et al. 2009; Hayes et al. 2012; Lv et al. 2013), and taxonomy (Hayes et al. 2012). However, studies on phylogeographic patterns and the demographic history of *Pomacea* ssp have been minimal. The aim of this study is to analyze genetic diversity and structure of *P. canaliculata* in 17 countries and *P. maculata* from 13 countries based on mtDNA COI sequences, which will provide a basis for effectively preventing and managing the spread of these two species.

Materials and methods

Data source

Pomacea belongs to the family Ampullariidae and is the largest of nine extant genera (Rawlings et al. 2007; Hayes et al. 2008). Due to their morphology there is considerable intraspecific variation and it is difficult to determine the true number of species (Cazzaniga 2002; Hayes et al. 2008). For example, the common name 'golden apple snail' also suggests that more than one species was recognized in Asia (e.g., Keawjam and Upatham 1990; Mochida 1991; Yipp et al. 1991). At the same time, it had even been referred to several species such as *Pomacea insularum* (Orbigny, 1835) and *P. canaliculata* (Lamarck, 1819) (Simpson et al. 1994; Roger 1996). Until recently, this snail was classified as two species, *Pomacea canaliculata* and *P. maculata* (as *P. insularum*, which is now a junior synonym of *P. maculata*) based on molecular, anatomy, and morphological data (Hayes et al. 2008; Hayes et al. 2012; Matsukura et al. 2013; Lv et al. 2013).

Due to the widespread issues with misidentification of *Pomacea* species, 146 sequences or haplotype sequences of *P. canaliculata* and 164 sequences or haplotype sequences of *P. maculata* were downloaded from GenBank (Suppl. material 1) and were classified as two species *P. maculata* and *P. canaliculata*, according to Rawlings et al. (2007), Deaton et al. (2007), Hayes et al. (2008), Tran et al. (2008), Matsukura et al. (2008), Jørgensen et al. (2008), Hayes et al. (2009), Song et al. (2010), Collier et al. (2011), Andree and López (2013), Lv et al. (2013), Matsukura et al. (2013), Mu et al. (2015), Ng et al. (2016), Letelier et al. (2016), Perez et al. (2017), Bocxlaer et al. (2017) (Suppl. material 1). COI sequences of *P. maculata* and *P. canaliculata* included 13 countries and 17 countries, respectively (Fig. 1).

Data analysis

The Clustal X1.81was used to align the mtDNA COI sequences of *P. canaliculata* and *P. maculata*, respectively (Thompson et al. 1997). DNASP 5.0 was used to analyze nucleotide composition, haplotype diversity (Hd) and nucleotide diversity (π) of the *P. canaliculata* and *P. maculata* for each country (Librado and Rozas 2009).

To test the phylogenetics of *P. canaliculata* (59 haplotypes) and *P. maculata* (42 haplotypes) COI haplotypes, MRBAYES v.3.2.2 was used to analyze a phylogenetic study using Bayesian inference (Ronquist et al. 2012). A comparison of 24 models of evolution was used to determine the initial model of evolution (HKY+G) in MRMODELTEST v.2.2. MRBAYES was run using 3,000,000 generations and six concurrent Markov Chains and 2 hot chains sampled at intervals of every 100 generations for a total of 30,000 trees. The stationarity of the log likelihood values was ensured using a 25% burn-in (7500 trees; Nylander 2004). Phylogenetic analyses included COI sequences for *Pomacea paludosa* (GenBbank accession numbers EU528477

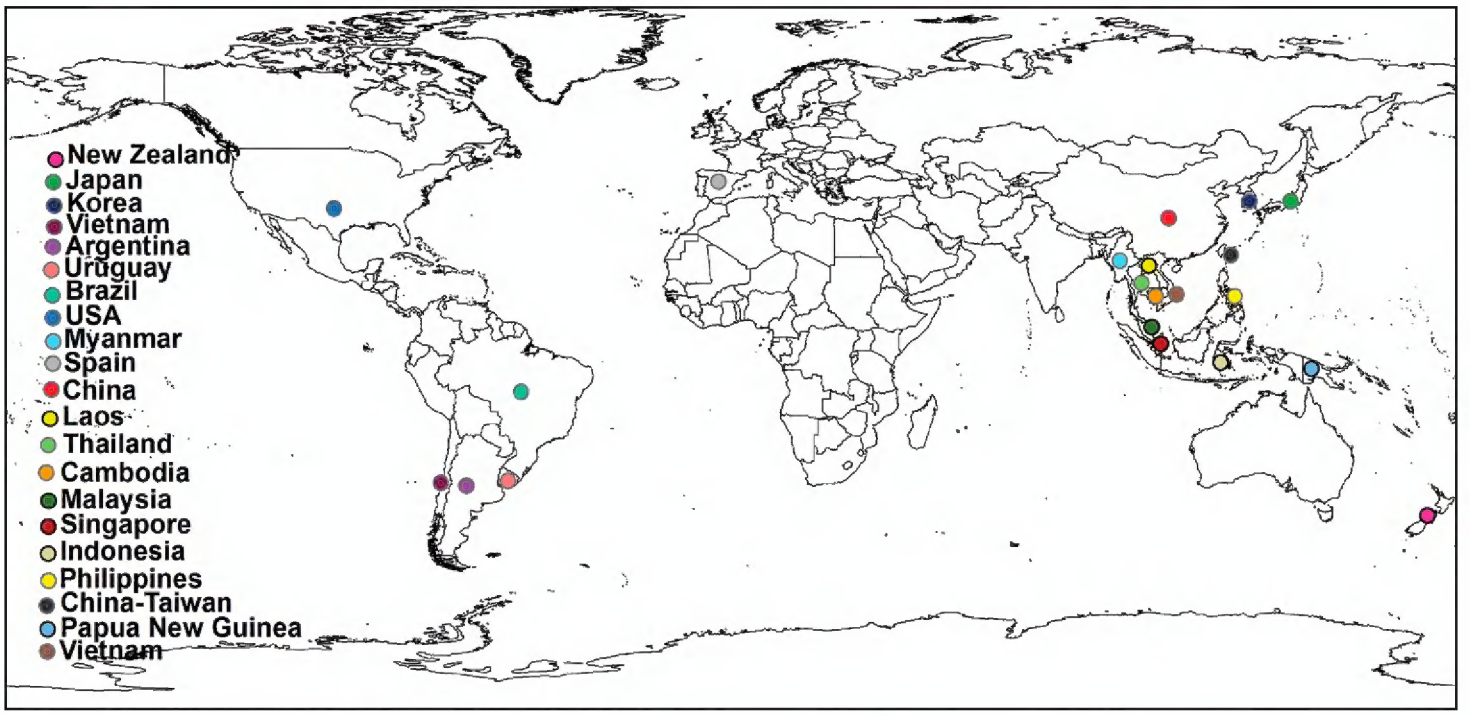

Figure 1. Distribution for *P. canaliculata* and *P. maculata* from 17 countries and 13 countries, respectively.

and EF514960) and *Pomacea diffusa* (GenBank accession numbers EU528564 and MF462141). As outgroups for the *Pomacea* dataset, COI sequences for *Pila conica* were used (GenBank accession numbers EU528588 and EU274570). NETWORK 4.5 was used to construct a haplotype network of the *P. canaliculata* (59 haplotypes) and *P. maculata* (42 haplotypes) COI haplotypes based on a 95% connection limit with gaps defined as missing data (Leigh and Bryant 2015).

A hierarchical analysis of molecular variance (AMOVA) was used to evaluate patterns of genetic structure in the COI dataset based on Arlequin 3.5 (Excoffier and Lischer 2010). The AMOVA was used to partition variance components to populations and to individuals within each collection location, where 1000 permutations were performed to test the significance of each pairwise *P. canaliculata* and *P. maculata* population comparison, respectively. Arlequin 3.5 was used to conduct a test for isolation by-distance by testing the significance of a correlation between pairwise Nei's D and geographic distance among country (Excoffier and Lischer 2010). A Mantel test was used to analyze the correlation computations between pairwise genetic and geographic distances between countries (Jensen et al. 2005). ArcMap GIS (ESRI) was used to measure the geographic distances within countries.

To examine deviations from neutrality of *P. canaliculata* and *P. maculata* for each country, Arlequin 3.5 was used to conduct Tajima's D and Fu's Fs tests (Excoffier and Lischer 2010). DNASP 5.0 was used to analyze mismatched distribution analysis (MDA) of *P. canaliculata* and *P. maculata* for each country. BEAST 1.4.7 (Drummond and Rambaut 2007) was used to compute the Bayesian Skyline Plot (BSP) (Drummond et al. 2005) analysis. BSP was used to reconstruct the effective population size fluctuations since the time of the most recent common ancestor (TMRCA). MCMC was run for 500 million steps, with sampling every 1000 generations and following a burn-in of the initial 10% cycles. TRACER 1.5 was used to conduct inspections of

the results and construction of the BSP (Rambaut and Drummond 2007). The model comparison function in TRACER 1.5 was used to assess the fit of the constant size population model and Bayesian Skyline coalescent models to the dataset.

Results

Phylogeographic patterns

Fifty-nine and 42 unique COI haplotypes of *P. canaliculata* and *P. maculata* were identified from 17 and 13 countries globally, respectively. The China-mainland and Brazil had the greatest variation with 24 haplotypes and 26 haplotypes in *P. canaliculata* and *P. maculata*, respectively (Table 1). Both haplotypes' diversity values of *P. canaliculata* and *P. maculata* at each population varied between 0 and 1.000 (Table 2). The greatest haplotype diversity of *P. canaliculata* occurred in Thailand, Philippines, Korea, Vietnam and Uruguay and *P. maculata* occurred in Vietnam and Thailand. Nucleotide diversity values of *P. canaliculata* ranged from 0 to 0.093 and *P. maculata* ranged from 0 to 0.029 (Table 2). The greatest nucleotide diversity of *P. canaliculata* occurred in Uruguay and *P. maculata* occurred in Brazil. In addition, the haplotype diversity of *P. canaliculata* and *P. maculata* of South America was greater than other continents (Table 2).

Phylogenetic analysis (Fig. 2a) showed that the clade of *P. canaliculata* had strong support (1.00). The 59 haplotypes of *P. canaliculata* were divided into four clades: 27 haplotypes of *P. canaliculata* from Argentina, USA, Papua New Guinea, Myanmar, Korea, Philippines, Vietnam, Malaysia, Japan, Chile, Thailand, Indonesia, China-Taiwan, and China formed one clade. Thirteen haplotypes of *P. canaliculata* from Philippines, Japan, Laos, Vietnam, and China formed a second clade. Eight haplotypes of *P. canaliculata* from Argentina, Japan, China-Taiwan, Uruguay, and Singapore formed a third clade, and eleven haplotypes of *P. canaliculata* from Argentina, Myanmar, Korea, China-Taiwan, Uruguay, Japan, and China formed the last clade.

Phylogenetic analysis (Fig. 2b) showed the clade of *P. maculata* had strong support (1.00). The 42 COI haplotypes of *P. maculata* were divided into two clades: 32 haplotypes of *P. maculata* from Brazil, Argentina, USA, Spain, Singapore, New Zealand, Vietnam, Malaysia, Thailand, Cambodia, and China formed one clade and ten haplotypes of *P. maculata* from Brazil, Argentina, USA, Japan, Korea, Thailand, and China formed a second clade.

The single haplotype network was produced based on COI haplotypes of *P. canaliculata* and *P. maculata* (Fig. 3a, b). The most frequent haplotypes of *P. canaliculata* and *P. maculata* were Hap4 and Hap17, which had 43 and 37 individuals. Forty-eight and 18 haplotypes were rare haplotypes in both *P. canaliculata* and *P. maculata* and just a single individual occurred in a country. As seen in the phylogeny of *P. canaliculata* and *P. maculata* (Fig 2a, b), the results of the haplotypes network also showed a similar geographic structure.

Table 1. Haplotype distributions of *Pomacea canaliculata* and *Pomacea maculata* from 17 countries and 13 countries, respectively.

Continent	Country(code)	*Pomacea canaliculata*		*Pomacea maculata*	
		Number of haplotype sequences or sequences	Haplotype distribution	Number of haplotype sequences or sequences	Haplotype distribution
Asia	China-mainland (CM)	31	Hap4, Hap30, Hap31, Hap33, Hap34, Hap35, Hap42, Hap43, Hap44, Hap45, Hap46, Hap47, Hap48, Hap49, Hap50, Hap51, Hap52, Hap53, Hap54, Hap55, Hap56, Hap57, Hap58, Hap59	6	Hap17, Hap27, Hap28
	China-Taiwan (CT)	4	Hap4, Hap34, Hap36	–	–
	Japan (JA)	22	Hap4, Hap5, Hap25, Hap30, Hap33, Hap34, Hap35, Hap36	6	Hap17, Hap37
	Philippines (PH)	13	Hap4, Hap20, Hap21, Hap22, Hap23, Hap24, Hap25, Hap26, Hap27, Hap28, Hap29, Hap30, Hap31	–	–
	Vietnam (VI)	2	Hap4, Hap30	2	Hap17, Hap42
	Thailand (TH)	2	Hap37, Hap38	2	Hap17, Hap37
	Myanmar (MY)	3	Hap4, Hap33	–	–
	Korea (KO)	2	Hap4, Hap34	1	Hap38
	Indonesia (IN)	3	Hap40, Hap41	–	–
	Laos (LA)	1	Hap30	–	–
	Malaysia (MA)	2	Hap4	1	Hap17
	Cambodia (CA)	–	–	1	Hap17
	Singapore (SI)	1	Hap36	1	Hap17
Oceania	Papua New Guinea (PNG)	2	Hap4	–	–
	New Zealand (NZ)	–	–	1	Hap17
Europe	Spain (SP)	–	–	9	Hap17
North America	United States (USA)	20	Hap4	66	Hap17, Hap37, Hap38, Hap39, Hap40, Hap41
South America	Uruguay (UR)	2	Hap32, Hap39	–	–
	Chile (CH)	4	Hap4	–	–
	Brazil (BR)	–	–	54	Hap1, Hap2, Hap3, Hap4, Hap5, Hap6, Hap7, Hap8, Hap9, Hap10, Hap11, Hap12, Hap14, Hap15, Hap16, Hap17, Hap18, Hap19, Hap20, Hap21, Hap22, Hap23, Hap24, Hap25, Hap26
	Argentina (AR)	32	Hap1, Hap2, Hap3, Hap4, Hap5, Hap6, Hap7, Hap8, Hap9, Hap10, Hap11, Hap12, Hap14, Hap15, Hap16, Hap17, Hap18, Hap19	15	Hap29, Hap30, Hap31, Hap32, Hap33, Hap34, Hap35, Hap36, Hap37, Hap38

The AMOVA results showed that 23.84% and 37.77% of the total genetic variance among countries was significant (F_{ST} =0.23836 and 0.37772, p<0.001, Table 3), respectively. Pairwise F_{ST} of *P. canaliculata* and *P. maculata* all ranged from -1.00 to 1.00 among countries (Table 4, Table 5). Pairwise gene flow of

Table 2. Genetic diversity of *Pomacea canaliculata* and *Pomacea maculata* from 17 countries and 13 countries of global based on COI sequences, respectively. Key: *H* = number of haplotypes, *H*d = haplotype diversity, π = mean nucleotide diversity.

Continent	Country	*Pomacea canaliculata*			*Pomacea maculata*		
		H	Hd	∏	H	Hd	π
Asia	China-mainland	24	0.968	0.030	3	0.700	0.023
	China-Taiwan	3	0.833	0.032	–	–	–
	Japan	8	0.835	0.029	2	0.600	0.009
	Philippines	13	1.000	0.028	–	–	–
	Vietnam	2	1.000	0.044	2	1.000	0.002
	Thailand	2	1.000	0.002	2	1.000	0.016
	Myanmar	2	0.667	0.029	–	–	–
	Korea	2	1.000	0.038	1	0	0
	Indonesia	2	0.667	0.001	–	–	–
	Laos	1	0	0	–	–	–
	Malaysia	1	0	0	1	0	0
	Cambodia	–	–	–	1	0	0
	Singapore	1	0	0	1	0	0
	Total	42	0.924	0.030	6	0.695	0.023
Oceania	Papua New Guinea	1	0	0	–	–	–
	New Zealand	–	–	–	1	0	0
	Total	1	0	0	1	0	0
Europe	Spain	–	–	–	1	0	0
	Total	–	–	–	1	0	0
North America	United States	1	0	0	5	0.708	0.006
	Total	1	0	0	5	0.708	0.006
South America	Uruguay	2	1.000	0.093	–	–	–
	Chile	1	0	0	–	–	–
	Brazil	–	–	–	26	0.970	0.029
	Argentina	19	0.924	0.023	10	0.914	0.018
	Total	21	0.933	0.031	36	0.978	0.030
Global Total		**59**	**0.853**	**0.029**	**42**	**0.893**	**0.022**

P. canaliculata and *P. maculata* all ranged from 0 to 249.75 among the countries (Table 4, Table 5).

Genetic differentiation as represented by pairwise genetic distance values of *P. canaliculata* ($P = 0.816$) and *P. maculata* ($P = 0.527$) among countries was not correlated with geographic distance, indicating that more geographically distant site combinations did not produce higher levels of genetic differentiation.

Demographic history

The mismatch distribution of analysis of pairwise differences was not significantly different from the expected distribution of the expanding population model based on COI sequences of *P. canaliculata* and *P. maculata* (Fig. 4a, b). Similarly there was a lack of statistical significance of Tajima's D test ($p < 0.01$), and non-significant Fu's FS ($p < 0.01$). Moreover, when all samples were pooled together, Tajima's D and Fu's FS test were again not significant ($p < 0.01$, Table 6). Additionally, the BSPs showed that *P. canaliculata* and *P. maculata* have had a stable historical population size with a small

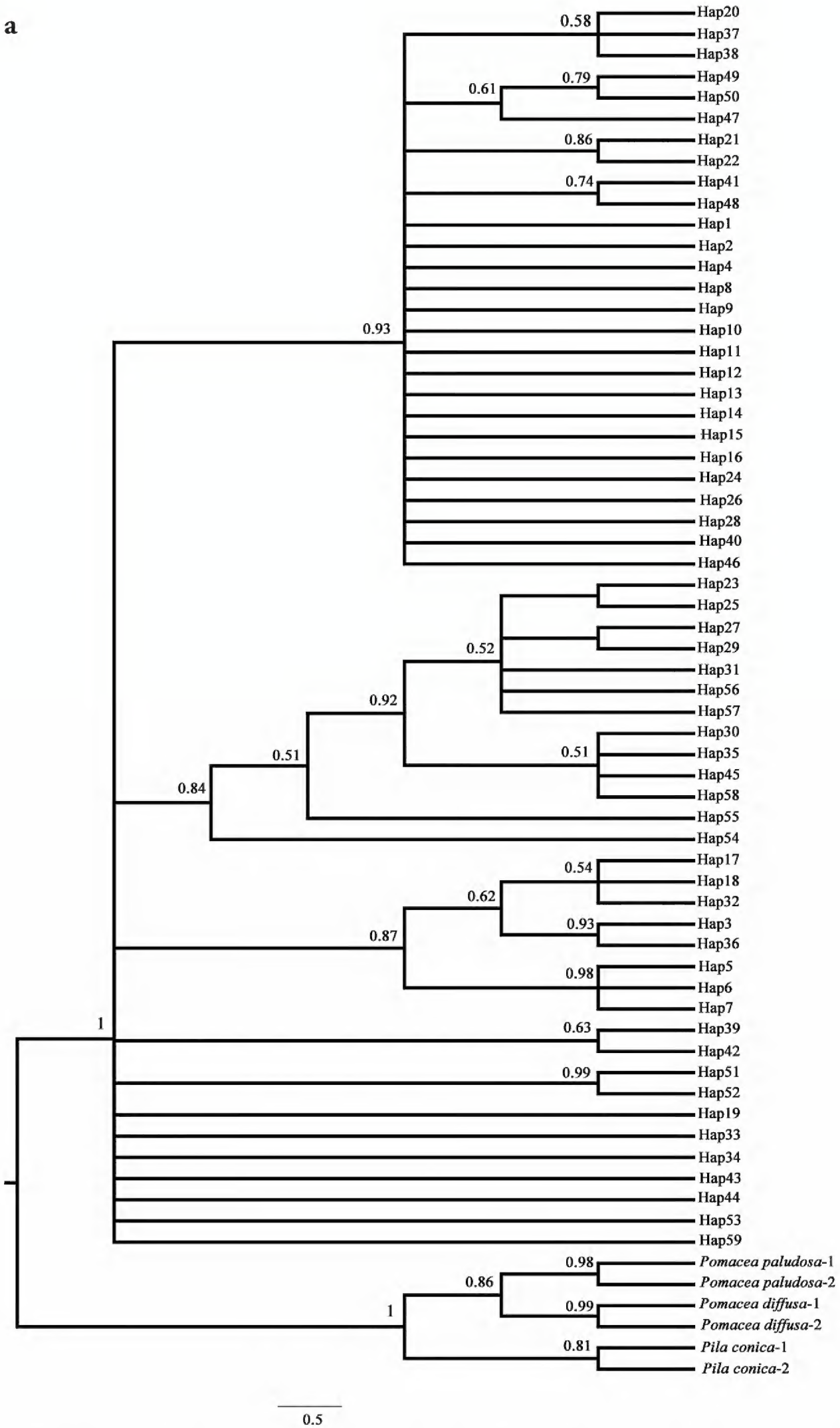

Figure 2. Phylogenetic analysis of the COI sequences for *P. canaliculata* (**a**) and *P. maculata* (**b**) from 17 countries and 13 countries using Bayesian Inference (BI), respectively.

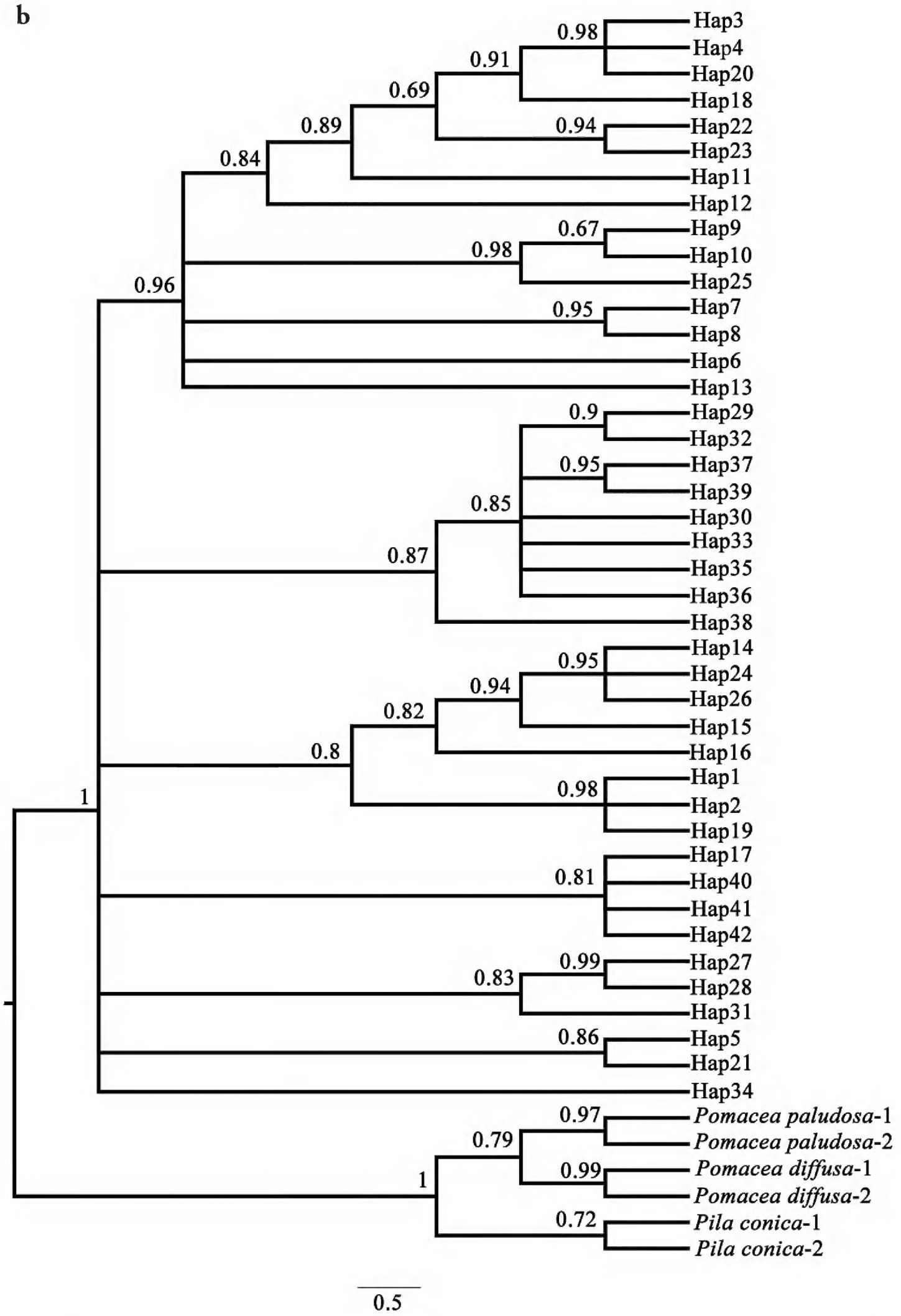

Figure 2. Continued. Phylogenetic analysis of the COI sequences for *P. canaliculata* (**a**) and *P. maculata* (**b**) from 17 countries and 13 countries using Bayesian Inference (BI), respectively.

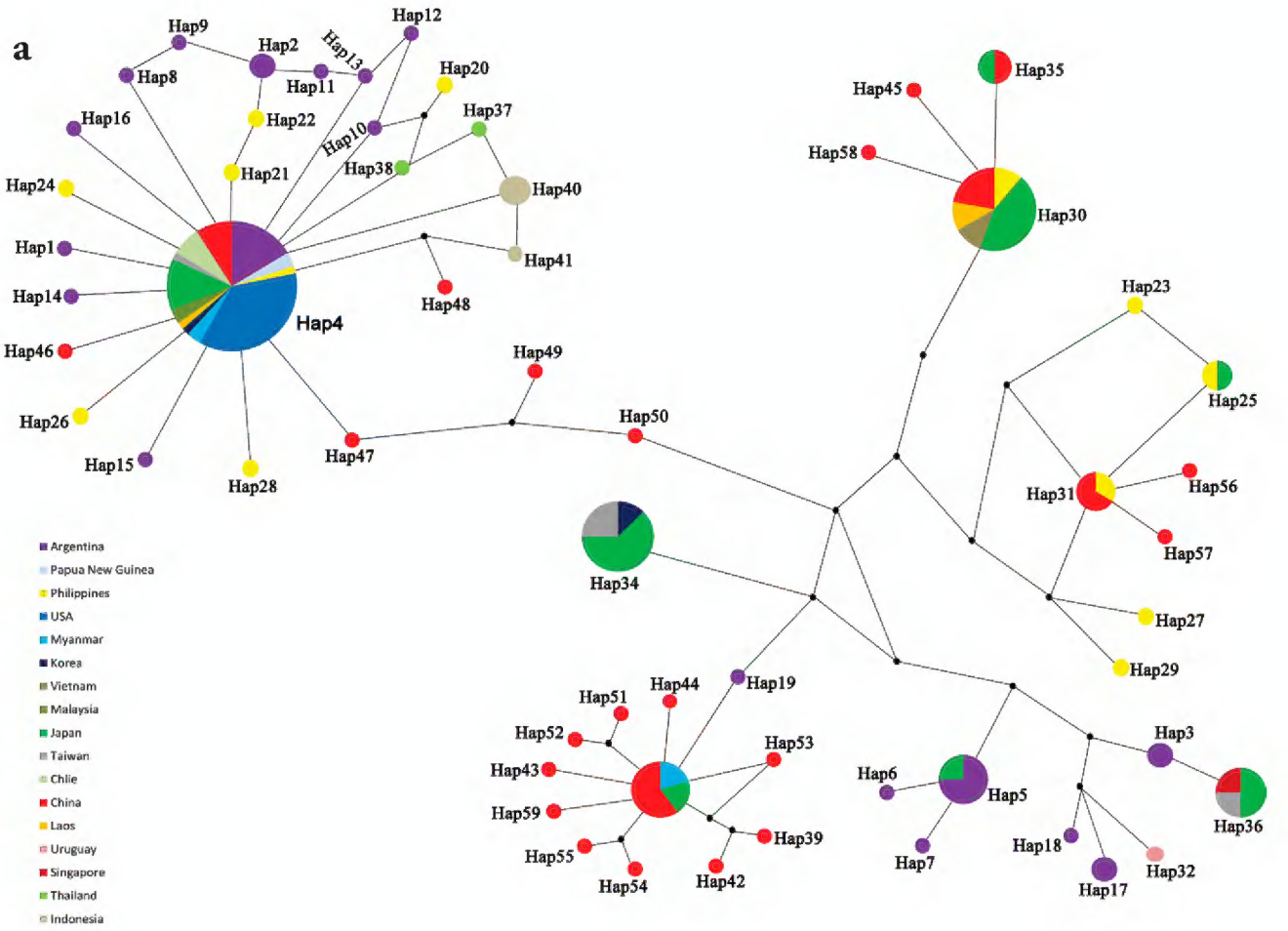

Figure 3. Haplotype network for *P. canaliculata* (**a**) and *P. maculata* (**b**) from 17 countries and 13 countries of global based on COI sequences. Each cross-hatched line represents one base-pair.

Table 3. Analysis of molecular variation (AMOVA) calculated from mtDNA COI sequences for *Pomacea canaliculata* and *Pomacea maculata* from 17 countries and 13 countries, respectively. All *F*-statistics were statistically significant (p<0.001). Key: F_{ST}=0.23836; F_{ST}=0.37772.

COI sequences		*P. canaliculata*				*P. maculata*		
Source of variance	**df**	**Sum of squares**	**Variance components**	**Percentage of variation**	**df**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among populations	17	322.04	1.78	23.84	12	380.34	2.72	37.77
Within populations	129	733.73	5.69	76.16	153	686.62	4.49	62.23
Total	146	1055.77	7.47	–	165	1066.95	1.73	–

Table 4. Analysis of genetic differentiation coefficient (F_{st}) (below diagonal) and gene flow (N_m) (above diagonal) calculated using COI mtDNA sequence data among 13 countries of *Pomacea maculata* in global. Bold type indicates statistical significance (α = 0.05). Country codes as in Table 1.

	CM	**JA**	**VI**	**TH**	**KO**	**MA**	**CA**	**SI**	**NZ**	**SP**	**USA**	**BR**	**AR**
CM		249.750	0.194	11.114	8.371	0.190	0.194	0.189	3.481	249.750	0.496	249.750	0.524
JA	0.001		0.084	49.750	20.583	1.284	0.077	0.071	0.444	31.000	0.762	249.750	0.796
VI	**0.563**	**0.749**		0.256	NA	NA	NA	NA	NA	NA	NA	0.534	NA
TH	0.022	0.005	**0.494**		3.917	0.255	0.254	0.251	1.474	49.750	0.633	249.750	0.615
KO	0.029	0.012	**1.000**	**0.060**		1.523	NA	NA	0.290	5.432	NA	0.406	0.454
MA	**0.568**	**0.163**	**1.000**	**0.495**	**0.141**		NA	NA	0.738	2.177	NA	0.159	NA
CA	**0.563**	**0.765**	**-1.000**	**0.496**	**1.000**	**1.000**		NA	NA	NA	NA	0.549	NA
SI	**0.570**	**0.779**	**-1.000**	**0.499**	**1.000**	**1.000**	**-1.000**		NA	NA	NA	0.534	NA
NZ	**0.067**	**0.360**	**-1.000**	**0.145**	**0.463**	**0.253**	**-1.000**	**-1.000**		NA	0.372	1.962	0.380
SP	0.001	0.008	**-1.000**	0.005	**0.044**	**0.103**	**-1.000**	**-1.000**	**-1.000**		1.139	249.750	1.124
USA	**0.335**	**0.247**	**1.000**	**0.283**	**1.000**	**1.000**	**1.000**	**1.000**	**0.402**	**0.180**		0.079	NA
BR	0.001	0.001	**0.319**	0.001	**0.381**	**0.611**	**0.313**	**0.319**	**0.113**	0.001	**0.759**		8.083
AR	**0.323**	**0.239**	**1.000**	**0.289**	**0.355**	**1.000**	**1.000**	**1.000**	**0.397**	**0.182**	**1.000**	0.030	

Table 5. Analysis of genetic differentiation coefficient (F_{st}) (below diagonal) and gene flow (N_m) (above diagonal) calculated using COI mtDNA sequence data among 17 countries of *Pomacea canaliculata* in global. Bold type indicates statistical significance (α = 0.05). Country codes as in Table 1.

	CM	**CT**	**JA**	**PH**	**VI**	**TH**	**MY**	**KO**	**IN**	**LA**	**MA**	**SI**	**PNG**	**USA**	**UR**	**CH**	**AR**
CM		24.750	0.004	2.002	27.528	0.059	0.212	NA	0.025	NA	10.620	0.235	0.422	0.640	41.417	0.581	0.059
CT	0.010		0.004	0.902	1.869	0.034	0.048	NA	NA	NA	0.244	0.146	0.194	0.391	249.750	0.411	0.000
JA	**0.986**	**0.984**		7.815	0.125	0.508	1.000	NA	0.330	NA	0.114	1.006	1.498	3.596	NA	3.596	0.496
PH	**0.111**	**0.217**	0.031		24.750	0.377	0.261	0.982	0.982	1.000	0.119	0.240	0.994	0.373	249.750	0.375	0.378
VI	0.009	**0.118**	0.666	0.010		NA	0.113	0.052	0.142	0.049	22.477	0.578	0.074	4.136	249.750	3.782	NA
TH	**0.809**	**0.881**	**0.330**	**0.399**	**1.000**		NA	NA	NA	NA	NA	NA	NA	NA	2.497	NA	NA
MY	**0.541**	**0.839**	**0.200**	**0.489**	**0.689**	**1.000**		0.505	0.256	0.499	NA	NA	0.164	NA	4.958	NA	NA
KO	**1.000**	**1.000**	**-1.000**	**0.203**	**0.829**	**1.000**	**0.331**		NA	NA	NA	0.496	0.164	0.496	NA	0.494	NA
IN	**0.910**	**1.000**	**0.431**	**0.203**	**0.638**	**1.000**	**0.494**	**1.000**		NA	NA	0.243	0.267	0.385	1.630	0.377	NA
LA	**1.000**	**1.000**	**-1.000**	**0.200**	**0.836**	**1.000**	**0.334**	**-1.000**	**1.000**		NA	0.499	0.168	0.499	NA	0.490	NA
MA	0.023	**0.506**	**0.686**	**0.678**	0.011	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**		NA	0.038	NA	249.750	NA	NA
SI	**0.515**	**0.632**	**0.199**	**0.510**	**0.302**	**1.000**	**1.000**	**0.335**	**0.507**	**0.334**	**1.000**		NA	NA	5.185	NA	NA
PNG	**0.372**	**0.563**	**0.143**	**0.201**	**0.772**	**1.000**	**0.604**	**0.604**	0.484	0.598	0.869	1.000		0.291	83.083	0.289	0.093
USA	**0.281**	**0.390**	**0.065**	**0.401**	**0.057**	**1.000**	**1.000**	**0.335**	**0.394**	**0.334**	**1.000**	**1.000**	0.462		62.250	NA	NA
UR	0.006	0.001	**-1.000**	0.001	0.001	0.091	0.048	**-1.000**	**0.133**	**-1.000**	0.001	0.046	0.003	0.004		62.250	2.438
CH	**0.301**	**0.378**	**0.065**	**0.400**	**0.062**	**1.000**	**1.000**	**0.336**	**0.399**	**0.338**	**1.000**	**1.000**	**0.464**	**1.000**	0.004		NA
AR	**0.809**	**1.000**	**0.335**	**0.398**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.729**	**1.000**	0.093	**1.000**	

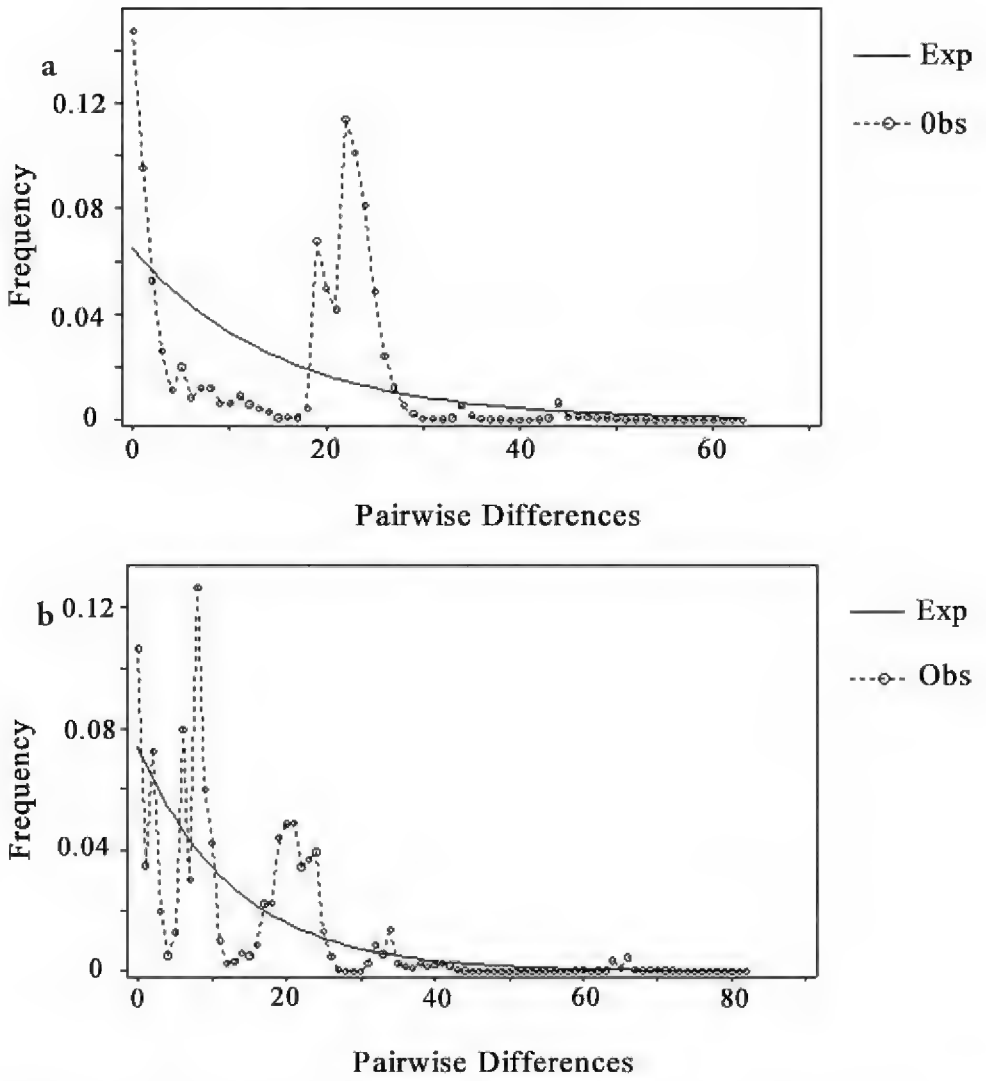

Figure 4. Mismatch distribution analysis (MDA) for *P. canaliculata* (**a**) and *P. maculata* (**b**) from 17 countries and 13 countries based on COI sequences.

recent expansion event occurring between 100,000 – 150,000 and 150,000 – 200,000 years ago, respectively (Fig. 5a, b). However, the model comparison analysis showed that constant population size was the best fit for the model of the data set, suggesting that there was not much support for the recent expansion trend.

Discussion

Phylogeographic patterns

Genetic diversity is an important basis for evaluating the status of population resources, which plays an important role in adaptations to habitat changes and maintenance of long-term survival and evolution (Freeland et al. 2011; Liu and Yao 2013; Liu et al. 2017). Invasive bivalves and *Pomacea* snails are typically r-selected species, which means they are adapted to colonize a wide range of aquatic environments and exhibit high fecundity, rapid growth, and broad physiological tolerances to several abiotic factors. In this study, *P. canaliculata* and *P. maculata* had high genetic diversity (0.853 and 0.893), which indicates adaptations to enable colonization of a wide range of

Table 6. Neutrality tests for *P. canaliculata* and *P. maculata* from 17 countries and 13 countries based on mtDNA COI sequences, respectively. Bold type indicates statistical significance (P < 0.01).

Country	*P. canaliculata*			*P. maculata*		
	Pi (%)	**Tajima's D**	**Fu's FS**	**Pi (%)**	**Tajima's D**	**Fu's FS**
China-mainland	15.13	0.16	-3.98	13.40	-1.15	4.50
China-Taiwan	16.33	0.34	3.47	–	–	–
Japan	14.54	0.70	6.33	5.40	2.20	5.78
Philippines	13.98	1.38	-4.39	–	–	–
Vietnam	22.00	0	3.09	1.00	0	0
Thailand	1.00	0	0	9.00	0	2.20
Myanmar	14.67	0	4.86	–	–	–
Korea	19.00	0	2.94	0	0	0
Indonesia	0.67	0	0.20	–	–	–
Laos	0	0	0	–	–	–
Malaysia	0	0	0	0	0	0
Cambodia	–	–	–	0	0	0
Singapore	0	0	0	0	0	0
Papua New Guinea	0	0	0	–	–	–
New Zealand	–	–	–	0	0	0
Spain	–	–	–	0	0	0
United States	0	0	0	3.74	1.72	4.73
Uruguay	47.00	0	3.85	–	–	–
Chile	0	0	0	–	–	–
Brazil	–	–	–	16.80	0.44	-0.46
Argentina	11.53	-0.02	-2.30	10.65	-2.37	0.17
Total	14.46	-0.79	-13.49	12.93	-1.24	-2.61

habitats. However, genetic diversity of ancestral populations (Brazil, Argentina, etc.) was higher than the recently established populations (Singapore, Papua New Guinea, New Zealand, Spain, etc.). The low haplotype diversity may be attributed to:

1) possible genetic exchange, genetic bottlenecks and genetic drift (Matsukura et al. 2013; Shirk et al. 2014), which could be the result of the initial founder event that led to speciation in *P. canaliculata* and *P. maculata* (Freeland et al. 2011);
2) the introduction of alien species to a non-native location may not be directly from the native range, but from a successful invasive population elsewhere, which could be the result of the bridge-head effect (Eric et al. 2010);
3) sample numbers of *Pomacea* for each country were significantly different for this study, so further genetic analysis is needed to clarify this (Hayes et al. 2008);
4) growth and reproduction of *P. canaliculata* and *P. maculata* are closely related to water temperature: many studies showed that the snail was not adapted to low temperatures (Andree and López 2013; Byers et al. 2013; Hayes et al. 2015; Bernatis et al. 2016). In addition, climate change (Byers et al. 2013) and environment factors such as pH (Byers et al. 2013), calcium carbonate (White et al. 2007; Perlman 2016), dissolved oxygen (Seuffert and Martín 2009), salinity (Verbrugge et al. 2012; Martin and Valentine 2014) may affect their life history.

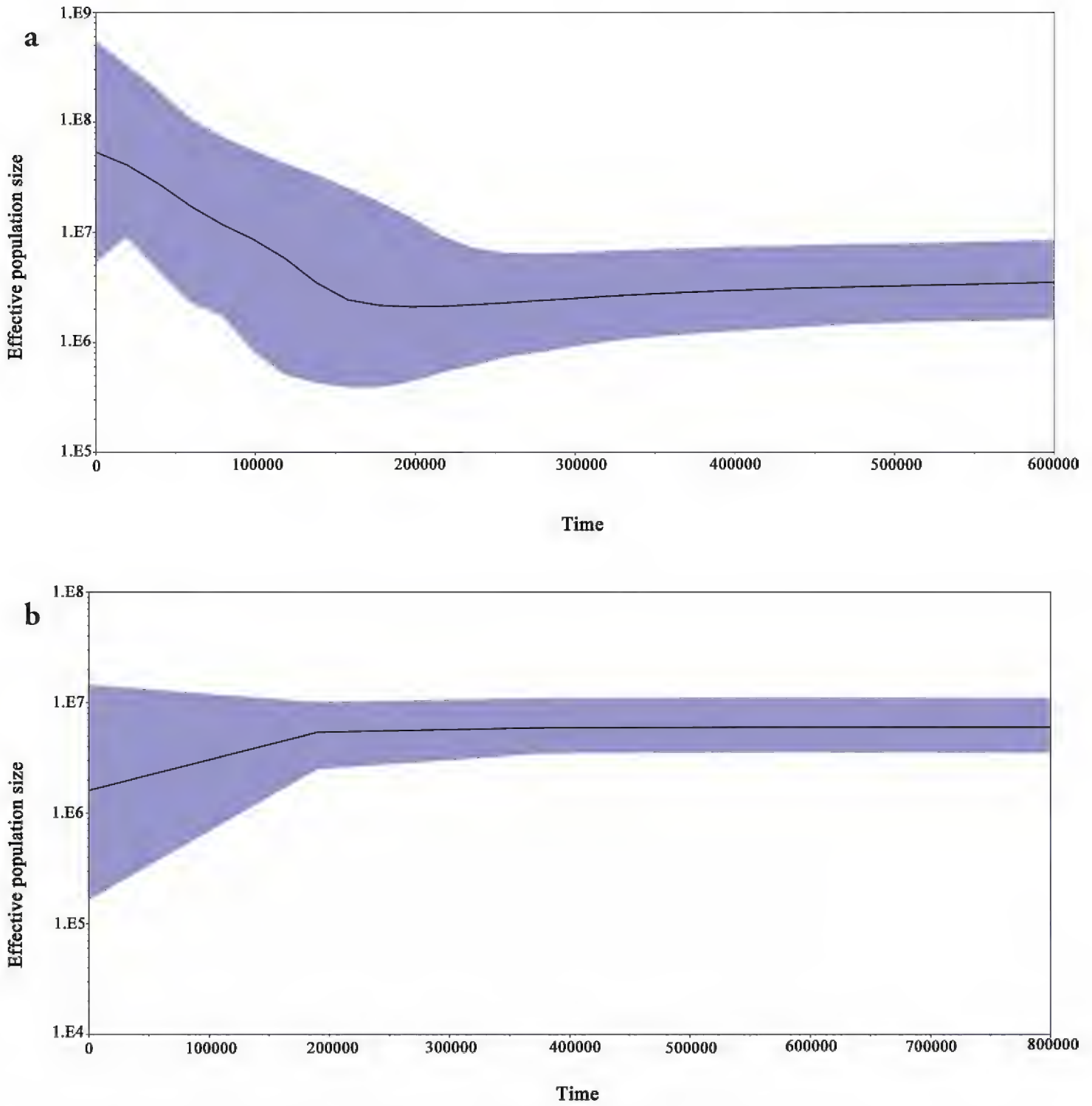

Figure 5. Bayesian skyline plot for *P. canaliculata* (**a**) and *P. maculata* (**b**) from 17 countries and 13 countries reconstructing the population size history using an evolutionary rate of 2.0×10^{-8} substitutions/site/year.

5) genetic diversity of *P. canaliculata* and *P. maculata* is related to human factors. *Pomacea* was introduced to many countries as food, but its economic potential was overestimated. Some regions, such as North America and Europe, attached great importance to the prevention and management of invasive species, which made it difficult for them to survive (Hayes et al. 2015; Joshi et al. 2017).

The geographic structure among countries using the mtDNA COI dataset was somewhat ambiguous. The phylogenetic analysis showed 59 haplotypes of *P. canaliculata* divided into four clades. These results also indicated a complicated pattern of introduction into non-native countries. The somewhat ambiguous genetic structures that may be attributed to *Pomacea* were introduced to many areas where they are consumed as food (Lv et al. 2009a; Hayes et al. 2008; Lv et al. 2013; Joshi et al. 2017).

Genetic differentiation was high in different countries hosting *P. maculata* and *P. canaliculata*. In addition, estimates of gene flow (N_m) were generally low (i.e., N_m <1). The higher level of genetic differentiation and low gene flow may be attributed to:

1) geographical isolation as an important factor that affects distribution patterns and genetic structure of species (Hayes et al. 2008; Lv et al. 2013). *Pomacea* are amphibious and show a preference for shallow ditches and ponds surrounding human settlements in which the water habitat is relatively isolated (Cowie 2002; Hayes et al. 2012; Joshi et al. 2017);
2) the dispersal ability of *Pomacea* is relatively low and its activity range is limited (Cowie 2002; Hayes et al. 2012; Joshi et al. 2017), which may lead to a gradual reduction of gene flow, and resulting in genetic differentiation among populations;
3) introduced populations usually experience a bottleneck if founded by a few individuals, and their genetic variability is expected to decrease in the newly colonized range (Dlugosch and Parker 2008; Hayes et al. 2008; Lv et al. 2013; Joshi et al. 2017).

In theory, an increase of geographic distance should correlate with a gradual reduction of gene flow, resulting in genetic differentiation among populations, i.e., isolation-by-distance (Hurtrez-Boussès et al. 2010; Husemann et al. 2012). However, genetic differentiation as represented by pairwise genetic distance values of *P. canaliculata* (P = 0. 816) and *P. maculata* (P = 0. 527) among countries was not correlated with geographic distance, which indicated more geographically distant site combinations did not produce higher levels of genetic differentiation.

Demographic history

The mismatch distribution analysis and neutrality tests showed that *P. maculata* and *P. canaliculata* across 17 and 13 countries, respectively, did not have recent population expansions. These analyses also indicated that the population dynamics of *P. maculata* and *P. canaliculata* are quite stable. This is not a surprising result because *P. maculata* and *P. canaliculata* are typically r-selected species and widely distributed in many countries. In addition, the stable historical population size with a small recent expansion may be attributed to:

1) the glacial period has had an important influence on the spatial distribution pattern and genetic structure of species (Clark and Mccabe 2009). During the Quaternary glacial period there were many glacial processes in the high and middle latitudes of the world due to the decrease of global temperature, which might have contributed to the *Pomacea* populations experiencing a small recent expansion in South America (Per et al. 2017);
2) possibly a constant population size was the best fit for the model to the data set, suggesting that there was not much support for the recent expansion trend.

Conclusions

Biological invasions are of growing concern regarding the conservation of global biodiversity (Lv et al. 2013). In recent decades, there has been a dramatic increase in species introductions with the increasing travel and trade associated with rapid economic globalization (Blumental 2006). *Pomacea* is a successful invader which possesses many characteristics, such as high reproductive capability and dietary flexibility, rapid growth rate, and strong resistance to environmental conditions (Estebenet and Martín 2002; Cowie 2002; Boland et al. 2008). Currently, *Pomacea* show a tendency to expand in many countries (Rawlings et al. 2007; Hayes et al. 2008; Byers et al. 2013; Lv et al. 2013). In this study, *P. canaliculata* and *P. maculata* showed high genetic diversity, significant genetic differentiation, limited gene flow, and stable population dynamics among different countries.

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Supplementary material 1

List of all individual *Pomacea* spp. and outgroups used, and GenBank accession codes

Authors: Xiongjun Liu, Yu Zhou, Shan Ouyang, Xiaoping Wu

Data type: molecular data

Explanation note: List of all individual *Pomacea* spp. and outgroups used, and GenBank accession codes.

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